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Tumour necrosis factor alpha (TNF α) and alpha-Klotho (α KL) in children and adolescents with chronic kidney disease (CKD)

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Abstract

Introduction: Chronic kidney disease (CKD) in children, despite the progress in science and technology, is still a serious challenge. Early CKD detection gives a chance of early therapeutic intervention and lowering the progression of the disease. According to several publications indicating the possible use of alpha-Klotho (α KL) and tumour necrosis factor alpha (TNF α) for the early detection of the disease in adults, an attempt was made to evaluate their usefulness in the paediatric population.

Material and methods: The study group consisted of 42 patients with CKD with a mean age of 10.7 years (18 girls and 24 boys). The control group involved 21 healthy children with a mean age of 8.4 years (11 girls and 10 boys). Anthropometrical parameters and blood pressure were taken and routine biochemical tests were performed in the whole group. The concentrations of TNF α and α KL in serum and urine were determined by enzyme immunoassay.

Results: Children from the CKD group showed a statistically significant difference in serum TNF α and α KL in comparison to the control group. There was no significant relationship between the evaluated markers and sex, presence of hypertension, or proteinuria in the children. The mean α KL serum concentration was higher in patients on dialysis compared to the group of conservatively treated children, whereas the values of TNF α in serum and urine, as well as the α KL in urine, did not differ significantly in these groups. A significant positive correlation was found between serum α KL concentration and serum creatinine, but there was no other correlation between serum α KL or TNF α concentration and any of the measured anthropometric and laboratory parameters.

Conclusions: Serum TNF α and α KL levels in children with chronic kidney disease, although being statistically different compared to the group of healthy children, except for the correlation of serum α KL and creatinine, showed no other correlations to the most parameters used for chronic kidney disease evaluation including, eGFR. Their usefulness in the early detection of kidney dysfunction in children was not proven. (*Endokrynol Pol* 2021; 72 (6): 625–633)

Key words: children; chronic kidney disease; TNF alpha; alpha Klotho; renal replacement therapy

Introduction

Chronic kidney disease (CKD) is a serious health problem worldwide, with increasing incidence and prevalence. Progressive kidney damage with a subsequent loss of glomerular filtration function ultimately results in the initiation of renal replacement therapy (RRT) [1]. The epidemiology of CKD in children originates mostly from the data available of stage 5 CKD patients on renal replacement therapy (RRT), which causes data on the scale of the problem in the paediatric population to be underestimated. In the global perspective, the prevalence of chronic kidney disease stage 2 or

lower is reported to be approximately 55–60 per 1 million of age-related population, with an approximate mean incidence of 11–12 cases per year per million, according to European researchers [2, 3]. The dominant causes in children are congenital kidney and urinary tract defects (CAKUT) and glomerulonephritis. Reports draw attention also to the growing problem of obesity and diabetes in the paediatric population, predicting an increase in the prevalence of CKD [4]. This problem also concerns children born prematurely or born with low body weight [5].

Symptomatology of CKD differs depending on the stage of the disease. In the initial stages the phenom-



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enon of glomerular hyperfiltration occurs, and the treatment consists of pharmacological nephroprotection, regulation of acid-base and water-electrolyte balance disorders, the use of a diet with reduced protein intake, and an adequate fluid balance. The uremic state develops, in which toxic metabolism products accumulate in the blood, resulting in a life-threatening biochemical and immunological dysregulation requiring renal replacement therapy [6, 7].

α KL is a single-pass transmembrane protein expressed mostly in the kidney, choroid plexus, and parathyroid gland. It occurs in two forms: transmembrane and soluble. Membrane α KL acts as co-receptor for fibroblast growth factor-23 (FGF23), enabling merging to the FGF receptor (FGFR) [8]. This FGF23- α Klotho-FGFR complex activates mechanisms crucial for maintaining mineral homeostasis and hormone regulations (PTH, FGF23, and 1,25-(OH)₂ vitamin D₃) in kidney, bone, intestine, and parathyroid gland, but also shows antiaging properties and downregulates insulin growth factor (IGF). The extracellular domain of α KL separated from the cell surface functions as an endocrine substance and is found in blood, urine, and cerebrospinal fluid. Its multiple renal and extra-renal functions such as antioxidation, modulation of renal ion channels, inhibition of apoptosis, anti-senescence, and suppression of Wnt-signalling and the renin-angiotensin-aldosterone system (RAA) have been studied by researchers in recent years [8–11]. Serum Klotho deficiency is found in both acute and chronic kidney injury [9, 12, 13], and it seems that the reduction of the α KL level might be related directly to the reduction in the number of functioning nephrons, which potentially qualifies α KL to the group of early markers of CKD progression. In view of the numerous data on Klotho's renoprotective effect, as well as those suggesting that the loss of this function may be among the causes involved in the development of renal disease [12, 14], learning the exact mechanisms of action within the kidneys requires careful analysis.

TNF α is a cytokine produced mainly by macrophages, but also many other cells of the monocytic lineage. There are two forms of TNF α : membrane-bound and soluble form, which is formed from the membrane form by the TNF α converting enzyme. TNF α acts through transmembrane receptor TNFR1, which is expressed in most mammalian tissues, and TNFR2 — highly regulated and expressed mostly in immune system cells [15, 16]. It induces several biological effects, such as apoptosis, cytotoxicity to neoplastic cell lines, stimulation of phagocytosis, induction of acute phase protein production in the liver, stimulation of the formation of fibroblasts and osteoclasts, increased body temperature, and others. The key role of this cytokine in the immunomodulation processes is also emphasized, which is the

basis for maintaining the body's immune homeostasis [15–17]. Many studies have shown the involvement of TNF α in the pathogenesis of both acute and chronic kidney disease [18–20]. However, taking into account the function in both inflammatory and immunoregulatory processes, it can be hypothesised that this cytokine is responsible not only for the pro-inflammatory effect, but also for the immunosuppressive effect in CKD. In adult studies, TNF α receptor levels are higher in the presence of impaired renal function, and they correlate with an increase in creatinine, and a decrease in eGFR and albuminuria [21–22]. It is believed that albuminuria itself may increase TNF α levels, but this issue has not been thoroughly investigated in children.

Most of the studies concerning α KL and TNF α were carried out in adults. Both of these proteins, due to the numerous functions that bind them to chronic kidney disease progression in adults, require separate analysis in the paediatric population.

The aim of our study was to evaluate the usefulness of TNF α and α KL measurements in serum and urine as biomarkers of early renal function decline in children diagnosed with CKD.

Material and methods

The study group (CKD) consisted of 42 patients aged 2 to 18 years with CKD (18 girls and 24 boys). The causes of CKD were CAKUT (45.2%), polycystic kidney disease (19%), tubulointerstitial nephritis (14.2%), glomerulonephritis (11.9%), nephronophthisis (4.7%), renal toxicity (2.5%), and tubulopathy (2.5%). The mean CKD duration was 5.9 \pm 4.5 years. Twenty-six children (62%) were diagnosed with hypertension. The children from the study group were treated pharmacologically with antihypertensive agents, iron formulas, vitamin D, calcium carbonate, and sodium bicarbonate. Ten children (23%) were treated with renal replacement therapy (RRT) using peritoneal dialysis (7 patients) or maintenance haemodialysis (3 patients). On admission, weight, height, and blood pressure were taken and routine biochemical tests were performed. Body mass index (BMI) was calculated using the formula [BMI = body weight (kg)/height (m)²]. Estimated glomerular filtration rate (eGFR) was calculated according to the Schwartz formula (mL/min/1.73 m²) [23]. Additionally, albuminuria (mg/day) using 24 h urine collection was evaluated.

The control group consisted of 21 healthy children aged 1 to 18 years admitted for procedures of one-day surgery or patients with monosymptomatic nocturnal enuresis (11 girls and 10 boys). All children presented no signs of kidney disease, had no symptoms of acute infection, and were in good clinical condition. Anthropometric measurements and the age of the study subjects and controls are presented in Table 1. The average age, weight, height, and BMI in the study group and the control group did not differ significantly. Both groups of patients were also examined for the assessment of FGF21 concentration in urine and blood serum. It was described in a separate paper [24], which is part of one research project.

The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice (resolution No. KNW/0022/KB1/110/I/12 of 03.12.2013), and written consent from parents or legal guardians, and/or the patients (above 13 years of age) was obtained.

Blood samples (3–5 mL) for laboratory tests were taken in Eppendorf tubes during an examination related to periodic control in the

Table 1. Clinical characteristics of evaluated children — children with chronic kidney disease (CKD) and control group (CG)

Parameter	CG			CKD			p-value CKD vs CG
	Total group (n = 21)	Girls (n = 11)	Boys (n = 10)	Total group (n = 42)	Girls (n = 18)	Boys (n = 24)	
Age [years]	8.4 ± 4.1	6.7 ± 3.6	10.3 ± 3.8	10.7 ± 4.6	10.6 ± 4.6	10.7 ± 4.7	NS
Height [cm]	128.5 ± 24.5	116.1 ± 21.8	142.2 ± 20.2	134.3 ± 26.5	132.1 ± 25.3	135.9 ± 27.8	NS
Height SDS	0.106 ± 0.96	-0.12 ± 1.19	0.35 ± 0.57	-1.6 ± 1.43	-1.68 ± 1.73	-1.54 ± 1.19	< 0.01
Body weight [kg]	31.9 ± 16.42	24.63 ± 12.82	40.0 ± 16.7	36.4 ± 20.6	32.1 ± 14.0	39.7 ± 24.1	NS
Body weight SDS	0.19 ± 1.15	-0.08 ± 1.36	0.49 ± 0.84	-0.98 ± 1.44	-1.22 ± 1.3	-0.8 ± 1.53	< 0.01
BMI [kg/m ²]	18.0 ± 3.3	17.2 ± 2.95	18.9 ± 3.7	18.5 ± 4.7	17.4 ± 2.6	19.3 ± 5.8	NS
BMI SDS	0.296 ± 1.05	0.2 ± 1.16	0.397 ± 1.02	-0.19 ± 1.11	-0.27 ± 0.95	-0.002 ± 1.23	NS

Data are presented as mean ± standard deviation; BMI — body mass index; SDS — standard deviation score; NS — non-significant

outpatient clinic in the morning (8:00–9:00). After centrifugation 1000^r for 15 minutes at 4°C, the serum was stored at -20°C until assayed. Urine samples (50–100 mL) were collected at the same time as the blood samples, and also kept at -20°C until evaluation (CKD group only). Concentration of albumin in 24-hour urine collection, as well as albumin and creatinine concentration in urine samples, was performed.

Determination of TNF α and α KL levels

Determination of serum and urine concentrations of TNF α and α KL was performed in the Chair and Department of Medical and Molecular Biology, Faculty of Medical Sciences in Zabrze, SUM in Katowice.

The concentration of the soluble α KL form was determined by ELISA (enzyme-linked immunosorbent assay) using commercially available kits from IBL International (Hamburg, Germany) according to the methods provided by the manufacturers by measuring extinction in a Quant Universal Microplate Spectrophotometer (Biotek Instruments Inc., Winooski, VT, USA). The sensitivity of the kit was 6.15 pg/mL, with 3.5% in-series error and 11.1% out-of-series error.

TNF- α concentration in blood serum and urine samples was determined by the enzyme immunoassay method from the TNF- α determination ELISA kit, Diaclone (Diaclone SAS, France), cat. No. 950.090.096, according to the manufacturer's instructions. Immuno-complex detection was based on the reaction with a recombinant anti-TNF- α molecule conjugated to horseradish peroxidase and TMB solutions as a substrate (TMB Substrate, slow kinetic, Sigma, USA). Absorbance readings were carried out using a Universal Microplate Spectrophotometer — μ QUANT from BIO-TEK INC (head office of Bio-Tek, California, USA), at a wavelength of 450 nm, and processing of results - using the KCJunior computer program (Bio-Tek, USA). The sensitivity of the set was 0.8 pg/mL, in-series error 5.43%, and out-of-series error 7.37%.

Statistical analysis

A database was prepared in a Microsoft Excel spreadsheet. For statistical calculations STATISTICA software licensed v. 10.0 was used (StatSoft Inc., Tulsa, USA). The level of statistical significance was assumed at $p < 0.05$. The arithmetic mean, median, minimum and maximum value, lower and upper quartile, and standard deviation were calculated. For all parameters, the compatibility of their distributions with a normal distribution was checked with Shapiro-Wilk test. For variables with normal distribution, the parametric test was used (t-test for independent variables in comparative analyses and Pearson's test for analyses of correlation). For other variables, nonparametric tests were applied (Mann-Whitney U-test for comparisons and Spearman's rank correlation test for analyses of correlation).

Results

The results of routine laboratory tests are presented in Table 2. Children with CKD had elevated mean daily urinary albumin excretion and albumin/creatinine ratio. Total serum protein and albumin levels remained within the normal range. Patients with proteinuria were significantly older (13.6 vs. 9.6 years/ $p < 0.05$). Mean eGFR in CKD group (with an average of 47.87 ± 20 mL/min/1.73 m²) and standardized levels of creatinine clearance were lowered showing no sex differences. Analysing the lipid profile, although the CKD group of girls presented increased mean total cholesterol, the average level of total cholesterol, HDL, and LDL cholesterol fractions were within the normal range and the triglyceride concentration was elevated in the whole CKD group. Among the CKD group, only two patients presented with obesity (BMI > 95 pc) and nine with increased values of systolic blood pressure (SBP > 97 pc). The mean MAP value was 79.1 ± 10.0 mmHg.

In children with CKD, both analysed parameters (TNF α and α KL) in serum showed a statistically significant difference in comparison with the control group (Fig. 1). Further analyses among patients from the CKD group showed no differences in the values of serum or urine α KL and TNF α levels in relation to sex and the presence of proteinuria in children. There was a difference for the mean α KL serum concentration, which was higher in patients on dialysis compared to the group of conservatively treated children, whereas the values of TNF α in serum and urine as well as the α Klotho in urine did not differ significantly in these groups (Fig. 2A–C).

A statistically significant positive correlation was found between serum α KL concentration and serum creatinine ($r = 0.4437$; $p = 0.01$) and urine α KL ($r = 0.5866$; $p = 0.01$). A negative correlation was found between serum TNF α and white blood cell count

Table 2. Biochemical parameters among children with chronic kidney disease (CKD) compared by sex

Parameter	Total group (n = 42)	Girls (n = 18)	Boys (n = 24)	p-value
Serum albumin [g/L]	44.02 \pm 6.25	42.8 \pm 7.9	44.8 \pm 4.7	NS
Total proteins [g/L]	69.3 \pm 7.58	68.23 \pm 8.7	70.1 \pm 6.7	NS
Parathyroid hormone [pg/mL]	184.3 \pm 278	296.9 \pm 400	104.5 \pm 83.2	p < 0.05
Total cholesterol [mmol/L]	4.8 \pm 2.2	5.49 \pm 3.18	4.29 \pm 0.72	NS
HDL-cholesterol [mmol/L]	1.3 \pm 0.39	1.165 \pm 0.38	1.40 \pm 0.38	NS
LDL-cholesterol [mmol/L]	2.74 \pm 1.98	3.43 \pm 3.02	2.31 \pm 0.69	NS
Triglycerides [mmol/L]	1.59 \pm 1.02	1.91 \pm 1.35	1.36 \pm 0.64	NS
Serum creatinine [mmol/L]	261.5 \pm 254	319.3 \pm 283.2	218.1 \pm 227.6	NS
Urea [mmol/L]	12.35 \pm 8.3	12.67 \pm 10.5	12.1 \pm 6.5	NS
HCO ₃ [mmol/L]	21.9 \pm 2.3	22.7 \pm 2.05	21.3 \pm 2.32	p < 0.05
Inorganic phosphate [mmol/L]	1.64 \pm 0.6	1.68 \pm 0.64	1.6 \pm 0.58	NS
Total calcium [mmol/L]	2.427 \pm 0.23	2.41 \pm 0.3	2.44 \pm 0.17	NS
White blood cells [G/L]	6.62 \pm 2.2	6.34 \pm 2.15	6.83 \pm 2.26	NS
Haemoglobin [g/dL]	12.17 \pm 2.35	11.93 \pm 2.85	12.4 \pm 1.94	NS
Daily urine albumin excretion [mg/24 h]	269 \pm 628	97.5 \pm 243.0	392.9 \pm 785.5	NS
Albuminuria [mg/L]	59 \pm 50	30.92 \pm 48.3	76.5 \pm 43.8	p < 0.01
Albumin/creatinine ratio [mg/g creatinine]	423.2 \pm 905	328.25 \pm 983	484.8 \pm 872	NS
Standardized creatinine clearance [mL/min/1.73 m ²]	49.33 \pm 24.15	48.75 \pm 25.4	49.8 \pm 24.0	NS
eGFR [mL/min/1.73 m ²]	47.87 \pm 20	50.56 \pm 22.6	46.26 \pm 20.17	NS

Data are presented as mean \pm standard deviation; HDL — high-density lipoprotein; LDL — low-density lipoprotein; HCO₃ — hydrogen carbonate; eGFR — estimated glomerular filtration rate. NS — non-significant. Significant boys vs. girls p < 0.05

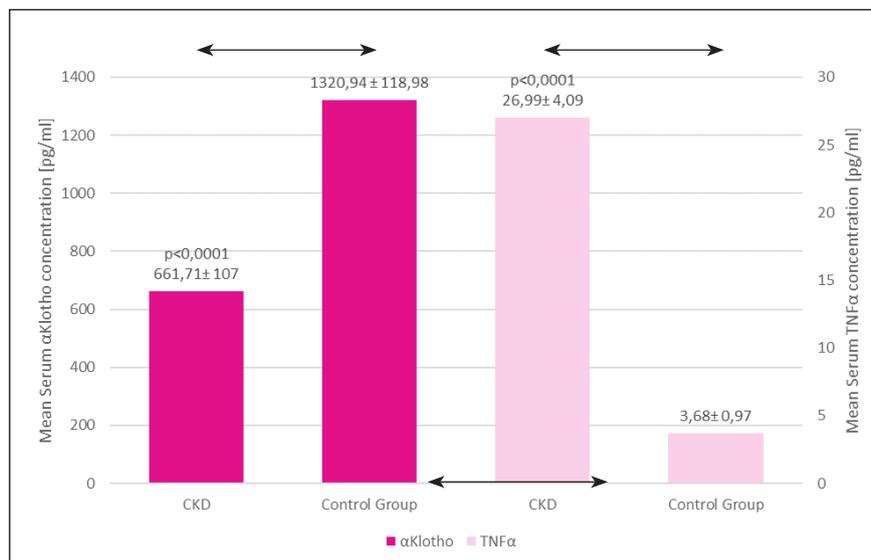


Figure 1. Mean serum α Klotho and tumour necrosis factor alpha (TNF α) concentration in chronic kidney disease (CKD) and in the control group

($r = -0.5202$; $p = 0.000$) and a positive correlation was found with urine TNF α ($r = 0.4753$; $p = 0.046$). There was no other correlation between serum α KL or TNF α concentration and any of the measured anthropometric and laboratory parameters. eGFR correlated negatively

with the albumin/creatinine ratio ($r = -0.4603$; $p = 0.01$), but no correlation with albuminuria was found. After adjusting to body mass index (BMI) and body surface area (BSA), we found positive correlations between α KL/BSA and triglycerides ($r = 0.32$; $p = 0.043$) and

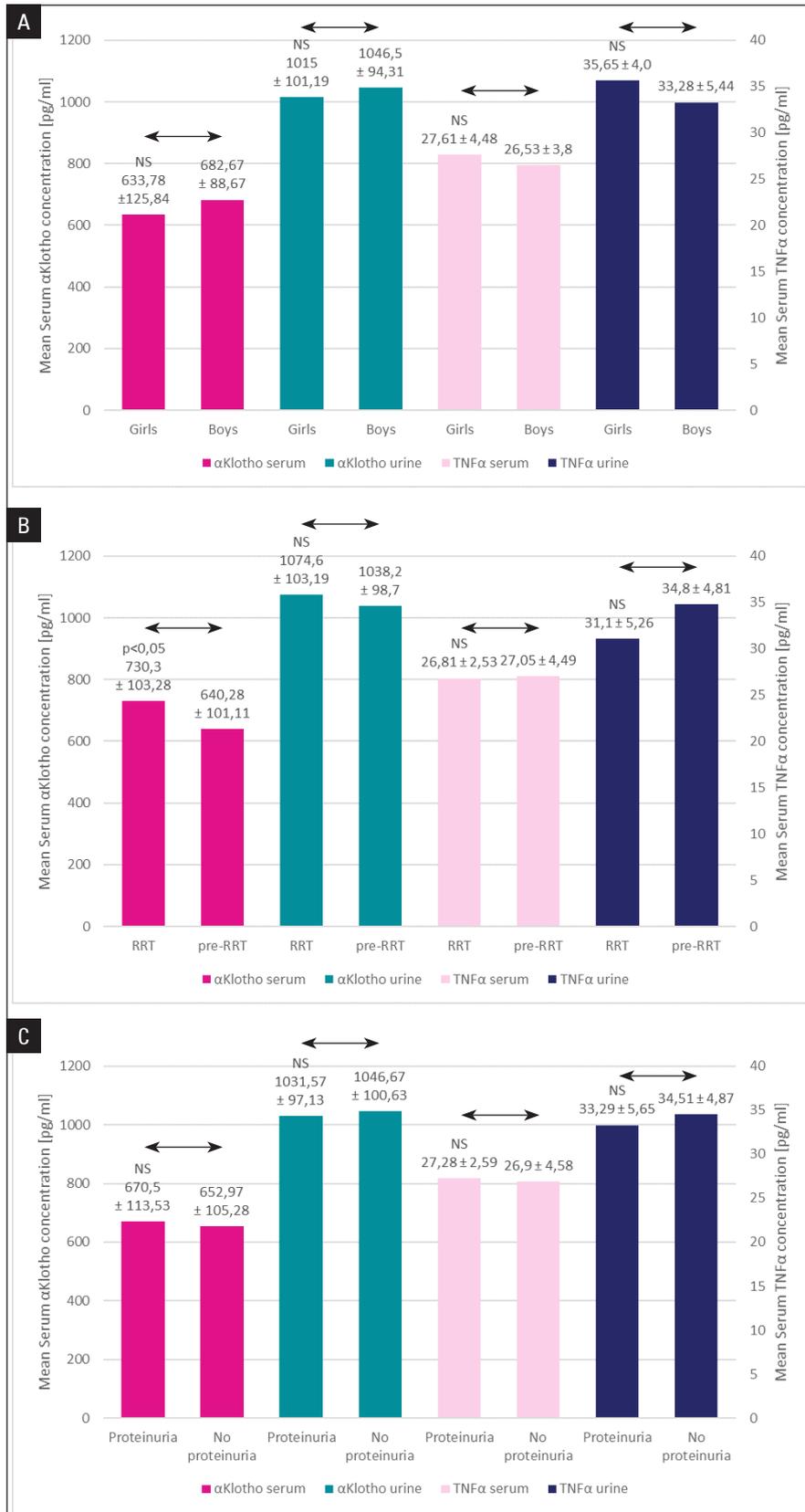


Figure 2A. αKlotho and tumour necrosis factor alpha (TNFα) levels in children with chronic kidney disease (CKD) depending on sex. **B.** αKlotho and tumour necrosis factor alpha (TNFα) levels in children on renal replacement therapy (RRT) and conservative treatment (pre-RRT). **C.** αKlotho and tumour necrosis factor alpha (TNFα) levels in children with chronic kidney disease (CKD) depending on proteinuria

a negative correlation between α KL/BMI and serum TNF α ($r = -0.5769$; $p = 0.012$).

Urine α KL concentration correlated positively with serum inorganic phosphate ($r = 0.399$; $p < 0.05$) and serum α KL. Urine TNF α correlated positively with total protein concentration ($r = 0.3687$; $p < 0.05$) and serum TNF α . No other statistically significant correlations for urine α KL or TNF α concentration were found (Tab. 3).

Discussion

The current study documents that inflammatory biomarker TNF- α is significantly elevated in patients with CKD compared to controls without CKD, and anti-ageing factor α KL significantly decreased, even in the paediatric population.

The manuscript of Hu et al. [9] explains the complicated mechanisms of KL renal and extra-renal actions. KL deficiency makes the kidney more susceptible to acute insults, delays kidney regeneration, and promotes renal fibrosis. The phenotypes of KL deficiency and CKD are very similar regarding gross phenotypes (body weight, growth retardation, life span, fertility), cardiovascular complications, or bone disease problems. There are some differences in blood biochemistry regarding mineral metabolism, including PTH (normal or low vs. elevated), vitamin D (elevated vs. decreased), and calcium level (slightly elevated vs. normal or low), but both states show elevated phosphate levels. We did not observe serious deviations in the parameters of calcium-phosphate metabolism in the studied patients; however, the entire group of patients had elevated PTH levels. Urinary KL and serum phosphate correlated positively, but no other correlation was found.

So far, it has been shown that many cell types have a very low baseline appearance of Klotho mRNA, but few of them express detectable amounts of Klotho protein. So, the effects caused by it are predominantly dependent on the soluble Klotho concentration [25]. Data from the literature show that in *in vitro* experiments soluble Klotho inhibited TNF α -induced expression of adhesion molecules ICAM-1, VCAM-1, and NF- κ B activation in human umbilical vascular endothelial cells (HUVECs). In that manner, the integrity of endothelium could be preserved and endothelial dysfunction could be prevented [26]. A study by He et al. further indicated that Klotho could significantly inhibit induced NF- κ B activation and the production of TNF α in monocytes [27]. In our study, although we confirmed low serum levels of α KL and high levels of TNF α , suggesting the lack of α KL inhibition, no correlation was found between the serum concentration of these compounds. Additionally, TNF α negatively correlated with the total white cell count.

Low levels of α KL protein were related to adverse cardiovascular outcomes, as examined by Sági et al. in adult patients with CKD on haemodialysis, including 24% of patients dialyzed due to diabetic nephropathy. Depressed soluble Klotho levels were shown in those patients, but the authors failed to detect their association with vascular calcifications or confirm their relationship with inflammatory indices [28]. We also did not confirm the relationship of Klotho concentration with variables describing blood pressure (SBP, DBP, MAP).

Inflammation and oxidative stress accelerate the decline of kidney function [29-32]. TNF is involved in both of these processes, being not only their promoter but also a factor that further aggravates these disorders as a result of the CKD progression. Odima et al. investigated paediatric patients on regular haemodialysis, confirming elevated oxidative stress biomarkers within this group, including TNF α , and their decrease after regular therapy by antioxidant medications for three months. The group of examined children presented a number of deviations in the biochemical parameters associated with the progression of CKD, including elevated PTH levels [33]. Among our studied patients we did not observe a significant increase in TNF α levels in end-stage renal disease, but the levels of calcium, inorganic phosphate, and PTH within this group were significantly higher than in the other stages of CKD.

A case-control study among 201 adult CKD patients, performed by Lee et al., suggested that TNF α was associated with the prevalence and severity of CKD. This phenomenon was independent of conventional CKD risk factors, the presence of cardiovascular disease, and used medications, including antihypertensive therapy. Additionally, they proposed that albuminuria could selectively elevate TNF α and/or this cytokine concentration may be increased as a result of decreased clearance of TNF α in patients with CKD [22]. Our observations, despite the presence of elevated levels of TNF α in the CKD group, showed no significance in the correlation of TNF α , both in urine and serum, to albuminuria, with a simultaneous positive correlation between serum TNF α and urinary TNF α , which seems to contradict the theory of impaired elimination.

Socha-Banasiak et al., in their study on children and adolescents with obesity, documented that Klotho levels were associated with body weight. Children with kidney diseases were excluded from that examination [34]. Our data showed no relationship of α KL levels with BMI, suggesting that in children with CKD the influence of kidney disease has a greater impact on Klotho levels than the presence of obesity. Due to TNF α participation in the development of obesity and its complications, this problem in the paediatric population was investigated by Goral et al. [35], who found

Table 3. Correlation between α Klotho and tumour necrosis factor alpha (TNF α) concentration and the results of chosen anthropometrical measurements and biochemical parameters among the group with chronic kidney disease (CKD)

Parameter	Serum α Klotho	Urine α Klotho	Serum TNF	Urine TNF
Body weight	r = 0.0183	r = 0.1443	r = 0.2662	r = 0.1021
Height	r = -0.0094	r = 0.0957	r = 0.2714	r = 0.0322
BMI	r = 0.0855	r = 0.2084	r = 0.1977	r = 0.1636
Age	r = -0.0058	r = 0.0675	r = 0.2945	r = 0.0379
SBP	r = -0.0459	r = 0.0705	r = 0.0736	r = -0.0755
DBP	r = -0.0368	r = 0.1124	r = -0.0411	r = -0.2131
MAP	r = -0.0298	r = 0.0989	r = 0.0053	r = -0.1686
Serum albumin	r = -0.0106	r = -0.0523	r = -0.0442	r = -0.1132
Total proteins	r = -0.0773	r = 0.0625	r = 0.1061	r = 0.3687
Parathyroid hormone	r = 0.2404	r = -0.049	r = -0.1306	r = -0.0478
Triglycerides	r = 0.1959	r = 0.3081	r = -0.092	r = 0.1576
Serum creatinine	r = 0.4437	r = 0.2267	r = -0.0089	r = -0.1227
Urea	r = 0.0648	r = 0.0777	r = 0.2285	r = 0.0267
HCO ₃	r = -0.0203	r = -0.1245	r = 0.1644	r = 0.1355
Inorganic phosphate	r = 0.2134	r = 0.399	r = -0.2229	r = -0.1532
Total Calcium	r = -0.1784	r = -0.0767	r = -0.0491	r = 0.1252
Haemoglobin	r = -0.1542	r = -0.2165	r = 0.1793	r = 0.0579
White blood cell count	r = -0.0972	r = -0.1106	r = -0.5202	r = -0.2721
Daily urine albumin excretion	r = 0.0289	r = 0.2263	r = -0.1114	r = -0.1121
Albuminuria	r = -0.2796	r = -0.089	r = -0.2339	r = -0.332
Albumin/creatinine ratio	r = 0.0853	r = 0.2886	r = -0.0412	r = -0.0638
Standardized creatinine clearance	r = 0.064	r = -0.0003	r = 0.1078	r = 0.0788
EGFR	r = -0.1886	r = -0.1172	r = 0.05	r = -0.1246

BMI — body mass index; SBP — systolic blood pressure; DBP — diastolic blood pressure; MAP — mean arterial pressure; HCO₃ — hydrogen carbonate; eGFR — estimated glomerular filtration rate. Significant correlation coefficients p < 0.

that the group of obese patients did not differ in terms of results of TNF α levels from the control group. The authors were able to establish only a significant positive correlation between TNF α and TG. In the publication of Azevedo et al. [31] an increased daily TNF α secretion was noted in malnourished children, with an additional clear surge of TNF α after contact with LPS, although decreased levels of TNF α in this group of children were also suggested by other authors. Body composition disorders or malnutrition are problems that often accompany patients with CKD. In our observations, all the parameters of lipid metabolism or total protein levels, as well as anthropometric parameters (weight, height, BMI), remained without a significant relationship with the TNF α levels, although the average values of cholesterol fractions fluctuated within high norms, and TG levels were increased in the whole study group.

Urine α KL and TNF α measurements did not show a significant correlation with proteinuria or the diagnosis of arterial hypertension. The same analysis carried out in terms of the need for dialysis or conservative treatment showed no differences in the levels of urine α KL and TNF α . These parameters did not correlate additionally with eGFR, creatinine, or the albumin/creatinine ratio. A positive correlation was noted between urinary and serum TNF α , urinary and serum α KL, urinary TNF α and serum total protein levels, and urinary α KL and serum inorganic phosphate levels. These observations are inconsistent. While the increase in phosphate levels may indicate a deterioration in nephron filtration, which may be reflected in excessive "loss" of α KL by the glomeruli, a similar mechanism is not applicable to TNF α loss. Jacobson et al. investigated the excretion of urinary oxidative stress biomarkers in children with CKD during several months of specialist observation [36]. The markers they studied, especially the baseline parameters, were associated with higher eGFR, lower proteinuria, and lower risk of renal replacement therapy. The authors noted that despite numerous reports on the role of oxidative stress in the development of CKD, their determination in urine may reflect the current kidney function rather than predict disease progression.

When analysing the activity of TNF α , attention is drawn to the key role of TNF α receptors TNFR1 and TNFR2, not only as key mediators of TNF α but also as elements of an independent pathway of renal injury and renal function decline [20]. It seems that the assessment of TNFR in children with chronic kidney disease may be a more accurate method of assessing the processes occurring in the course of chronic kidney disease than the analysis of the TNF α level itself, which certainly requires further studies in the paediatric population [37, 38].

A major limitation of the study is the small number of examined children with CKD, which is the effect of epidemiological conditions and could be solved by longer data collection or the organization of a multicentre study. Other limitation is the lack of evaluation of urine TNF α and α KL in healthy patients. Nevertheless, despite the difficulties in observing the paediatric population, each analysis seems to be an important voice in the discussion on better understanding of CKD as well as improving patient treatment.

Conclusions

Serum TNF α and α KL levels in children with chronic kidney disease, although being statistically different compared to the group of healthy children, except for the correlation of serum α KL and creatinine, showed no other correlations to most of the parameters used for chronic kidney disease evaluation, including eGFR. Their usefulness in the early detection of kidney dysfunction in children was not proven.

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